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STUDIES ON THE GLUTEN PROTEINS

Robert Charles Rose

Department of Field Crops

University of Alberta

April, 1934

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Robert Charles Rose

Department of Field Crops

A THESIS

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INTRODUCTION

Gluten is a term applied to the tenacious mass which is obtained when the starch is washed from a firm dough of wheat flour. The composition of such wet gluten depends largely on the physical properties of the contained proteins, but when the flour is made from high grade, hard red spring wheat, the wet gluten is approximately 28% protein, 5% starch and other non-protein solids and 67% water. The gluten proteins are generally considered to be two in number: gliadin, an alcohol soluble protein or prolamine; and glutenin, classified as a glutelin, and defined as being insoluble in all neutral solvents although soluble in dilute acids and alkalis.

Although the physical properties of gluten determine, to a large extent, the baking quality of the flour from which it was obtained, the recorded data on the physical and chemical properties of the gluten proteins are confined almost entirely to studies involving dispersion

in acids, alkalis and alcohol. As these solvents are known to denature or otherwise alter the physical properties of other proteins, such as albumins, it seems likely that they would affect the gluten proteins in a similar manner.

Glutenin, although defined as insoluble in neutral solvents, has recently been shown to disperse in urea solutions, and also, as reported in this paper, in sodium salicylate solutions. The use of sodium salicylate has one advantage over the use of urea in that the protein content of the dispersion can be more easily determined. These neutral solvents, however, are also known to denature other proteins, but the relative extent of their effect on gluten, compared with that of acids and alkalis, is not known. These neutral solutions have at least one advantage over ~~that of~~ acids and alkalis in that they permit a study of gluten in its normal isoelectric condition, and although they may cause denaturation, it seems probable that such secondary changes as incipient hydrolysis would be avoided in these dispersion media.

The first part of this investigation is concerned with the viscosity changes which occur in gluten dispersed in sodium hydroxide, acetic acid, urea and sodium salicylate solutions. The object was to obtain information regarding the properties of dispersed gluten, and also the changes which occur during storage in these dispersion media.

A knowledge of these changes might give an indication of the best reagent for dispersing gluten for either chemical or physical studies. Furthermore, the conditions favoring a reasonably constant, reproducible viscosity had to be determined before undertaking further studies such as the effect of heat treatment.

There is a considerable body of indirect evidence to show that the gluten proteins are altered physically by subjecting wheat or flour to elevated temperatures. Yet when a dispersion of either gluten, gliadin or glutenin, is heated there is no evidence of coagulation similar to that which occurs when albumins are heated. The second part of this thesis covers a viscometric study of gluten, dispersed in various media, and subjected to various heat treatments.

REVIEW

Viscosity. The most characteristic property of a lyophilic colloid, such as a gluten dispersion, is its extremely high viscosity. This property can be measured relatively easily, but the high values obtained cannot be explained in terms of the theories and equations which apply to the viscosity of true solutions. In order to explain their low fluidity it has therefore been necessary

to assume that the colloidal particles are either highly hydrated (16, 17) or interlinked and connected to form a micellar structure (28). Kraemer (21) reviewed the literature on the viscosity of lyophilic colloids and concluded that, although solvation in a specific chemical sense is undoubtedly a primary factor in determining the swelling and aggregation of particles, the high viscosities are probably due to the mechanical immobilization of the dispersion medium by the presence of bulky aggregates, or by elongated particles that increase resistance to shear through mutual entanglement. Lyophilic colloid solutions also differ from true solutions in that the observed viscosity varies with the rate of shear, high shearing stresses giving lower values. The viscosity coefficients of such material are often termed "apparent viscosities".

As different types of viscometers, and even similar types of different structural dimensions, have different rates of shear, it is obvious that comparable results can only be obtained with viscometers which are practically identical in size and shape. Moreover, a large range of viscosities cannot be measured in the same viscometer as the time factor, i.e. the rate of shear, varies appreciably. In view of these considerations the British Engineering Standards Association (8) has adopted a series of standard U-tube viscometers for the

determination of viscosity in absolute units, each one to be used only within a definite range. When such viscometers are used the viscosity can be justifiably and more conveniently expressed in absolute than in relative units, and this facilitates comparisons between the results obtained with viscometers covering different ranges. The poise is a large unit, but if the centipoise is used this objection cannot be raised as the viscosity of water at 20°C. is approximately one centipoise.

Properties of proteins in acids, alkalis and neutral solutions. Much of the present information and theory concerning the properties of native proteins has been derived from experiments with readily soluble proteins such as albumins and globulins. These proteins may lose their original solubility through the action of acids, alkalis, alcohols, certain salts and other reagents, heat, ultra-violet light and agitation, and this loss of solubility is termed denaturation. It is doubtful if the various forms of denaturation represent identical changes, but the classical theory of denaturation is that it is a chemical change followed by flocculation.

Osborne's (29) classical work on gluten led to the conclusion that it was composed of two proteins: an alcohol soluble fraction, gliadin; and an alkali soluble fraction, glutenin. Recent evidence (10, 18, 30), however, indicates that gluten can be fractionated into a series

of proteins, differing in chemical and physical properties.

Gluten dispersed in a relatively strong dispersing agent such as dilute alkali could hardly be expected to behave in a similar manner to the albumins. Heating the dispersion does not produce a coagulum, and although standing at room temperature for several days may yield a precipitate, this does not necessarily indicate true denaturation since the insoluble material may be the result of secondary changes in the protein.

Many workers have prepared glutenin after dispersion in alkali. Among the more recent, Hoffman and Gortner (19) used 0.5% sodium hydroxide and Larmour (22), 0.2% sodium hydroxide. Hoffman and Gortner used a Sharples supercentrifuge to remove the impurities, and they reported that the centrifuged material was practically water clear. Blish and Sandstedt (7) showed that, in the temporary dispersion of glutenin in alkali, there was always an irreversible alteration of the physical and chemical properties of the protein, the extent of the changes partially depending on the concentration of the alkali. Later these investigators (30) stated that alkali accomplished the complete dispersion of gluten protein in a short time, and the dispersions could be filtered water clear without loss of protein. On neutralization most of the protein could be recovered, but its original coherence and elasticity were lost.

In an attempt to find a more satisfactory dispersion medium for the preparation and purification of glutenin, Blish and Sandstedt (7) investigated the use of dilute acetic acid. In a later paper they (30) stated that wet gluten could be completely dispersed in acetic acid of almost any concentration. They used 0.05 to 0.1 N. On neutralization the gluten could be obtained in its original cohesive and elastic form and redispersion could be repeated several times without noticeably changing the physical properties of the gluten. This apparently harmless dispersion, in contrast to the destructive alteration by dilute alkali, was taken to indicate the superiority of acetic acid as a dispersing agent. The dispersions in dilute acetic acid could not be filtered, and some protein was thrown out by a Sharples supercentrifuge.

Further information on the action of acids and alkalis on proteins is reported by Svedberg and his associates who investigated the pH stability region of a number of proteins by means of the ultracentrifuge. Svedberg and Nichols (32) found carbon monoxide hemoglobin to be stable at least from pH 6.0 to 9.05. At pH 9.05 the hemoglobin molecule appeared to hold a monomolecular layer of water at its surface. Svedberg and Sjögren (33) found Bence-Jones protein to be stable from pH 3.5 to 7.5, but below pH 3.5 the molecules broke into a non-centrifugible

substance, and above pH 7.5 slight alkaline hydrolysis set in. Svedberg and Stamm (34) found that edestin was unstable on the acid side of its isoelectric point, there being definite dissociation at pH 3.6. Above its isoelectric point edestin was stable as high as pH 9.7 but dissociated at pH 11.3. Speakman and Hirst (31) found that wool keratin was stable at least from pH 4.0 to 7.0, but above and below this range it was less stable than at its isoelectric point. These results show that both acids and alkalis may affect the physical properties of proteins.

Loughlin and Lewis (27) found the viscosity of solutions of undenatured and of denatured egg albumin and oxyhemoglobin to be independent of pH provided that no flocculation occurred. They varied the pH of undenatured egg albumin from 1.97 to 7.61; of denatured, from 2.12 to 2.90; of undenatured 3% oxyhemoglobin from 4.58 to 8.21; and of denatured, from 2.38 to 5.07. The measurements on undenatured protein were made 10 to 15 minutes after adjusting the hydrogen-ion concentration. The viscosity of denatured protein could not be studied near its isoelectric point as precipitation occurred. Loughlin and Lewis (27) and Loughlin (26) concluded that no electro-viscous term need be taken into account in the case of egg albumin or oxyhemoglobin.

The neutral solvents known to disperse gluten include 30% urea solution which was used by Cook and

Alsberg (11) for the preparation of glutenin. Gortner, Hoffman and Sinclair (15) showed that a normal solution of potassium iodide would extract approximately 64% of the protein from wheat flours. Cook and Alsberg were unable to obtain complete dispersion of gluten in 30% potassium iodide, and consequently abandoned the use of lyotropic salts as dispersing agents for gluten. As far as the author is aware, the use of sodium salicylate solution as a neutral solvent for gluten has never been reported although Anson and Mirsky (2) showed that sodium salicylate would disperse denatured hemoglobin.

Regarding the extent of denaturation in these neutral reagents Cook and Alsberg (11) reviewed the literature on the denaturing effect of urea, and pointed out that there appear to be fewer progressive secondary changes in protein dispersed in urea solution than in acid or alkali solution. The dispersions in 30% urea solutions could not be filtered, but could be centrifuged in a Sharples supercentrifuge. Cook (10) studied the viscosity of an approximately 3% gluten dispersion in 30% urea solution stored at 5°C. after allowing 40 hours for complete dispersion. He showed that over a period of 101 hours after this initial 40 hours the viscosity remained essentially constant. This indicated that urea causes no further change in the protein after complete dispersion has occurred.

Anson and Mirsky (2) showed that when hemoglobin was allowed to stand in urea solution the viscosity increased as denaturation proceeded, but finally reached a high constant value when all the protein was converted into a form which was insoluble when the urea was removed, or as they suggested, when denaturation was complete. Later, these workers (5) showed that the addition to saturation of solid urea to viscous solutions of denatured egg albumin resulted in a lowering of the viscosity. They were of the opinion that this lowering was due to the solubility effect of urea and were there no such effect, the addition of urea would increase the viscosity.

Anson and Mirsky (2) also demonstrated that acid, alkali, potassium thiocyanate, potassium iodide and sodium salicylate, like urea all convert hemoglobin into an insoluble form, but even at its isoelectric point denatured hemoglobin can be dissolved, not only in concentrated urea solutions, but also in concentrated potassium thiocyanate, potassium iodide and sodium salicylate solutions.

It is evident that the dispersing agents used in this study, namely, dilute sodium hydroxide, dilute acetic acid, concentrated urea and concentrated sodium salicylate solutions, all denature proteins, and it cannot be said which one alters the properties of the gluten proteins least. In connection with the action of dispersing agents, Cook and Alsberg (11) expressed the opinion that if proteins,

such as glutenin, which are not dispersed by mild neutral solvents, obtained their insolubility from their state of aggregation, and if dispersion resulted in the breaking up of these aggregates, then all such proteins when dispersed would be denatured.

Heat denaturation. The generally accepted view of heat denaturation is that of Chick and Martin (9) who studied the heat coagulation of egg albumin. These investigators concluded that denaturation proper is a chemical process which in the heat coagulable proteins is followed by flocculation, a strictly colloidal phenomenon which takes place most rapidly at the isoelectric point of the protein. They consider it incorrect to speak of protein as having any particular "coagulation temperature", but rather that the process is a chemical reaction with a very high temperature coefficient.

Bancroft and Rutzler (6) reported that the extraction of aqueous solutions of egg whites with ether prevented the coagulation of egg albumin by alcohol and heat. They concluded that ether prevented denaturation by some colloidal mechanism and that denaturation was a colloidal phenomenon.

Lewis (23, 24) found that the velocity of heat denaturation of oxyhemoglobin and of egg albumin was minimal when the hydrogen and hydroxyl ion concentrations were sensibly equal. He (25) also found that high salt

concentrations decreased the rate of denaturation of oxyhemoglobin. The reaction was unimolecular. The critical increment was 77,500 calories in the case of oxyhemoglobin and 130,000 calories in the case of egg albumin. He suggested that a possible mechanism consists of a localized hydrolysis of relatively labile links situated at various points on a large, heavily hydrated molecular unit.

Loughlin and Lewis (27) studied egg albumin and oxyhemoglobin and showed that the viscosity of the (heat) denatured protein was higher in each case than that of the undenatured. They showed by dilatometer measurements that no change in net volume occurred on denaturation. Loughlin and Lewis (27) and Loughlin (26) concluded that denaturation involves a distension of each individual protein unit, accompanied by an increase in the amount of water associated therewith, so that the volume of each unit is approximately doubled. This conclusion, as they pointed out, is in accordance with the well known fact that heat denaturation will not occur in the absence of water. Loughlin and Lewis (27) stated, "Briefly the process of denaturation from the physical standpoint consists in a change from the molecular to the micellar state (without aggregation, however) on the part of each protein unit."

Although, for many years, denaturation was considered an irreversible process, this view is not now

generally held. Anson and Mirsky (3) concluded that denatured globin could be restored to its original state, and (4) that the denaturation of serum albumin could be reversed. Bancroft and Rutzler (6) stated that immuno-biological tests for species specificity and isoelectric point measurements have shown that coagulated and repeptized egg white sols are identical with the original.

Geddes (12, 13, 14) reviewed the literature on the effect of heat on wheat and wheat flour. The effect is of economic importance as mild heat treatment may improve the baking quality of flour whereas excessive heat treatment ruins the flour. He (14) concluded that the influence of heat as reflected by baking quality is detrimental to the gluten, but markedly decreases the deleterious effects of the germ constituents of the flour.

Alsberg and Griffing (1) studied the swelling of gluten disks in dilute acid after 30 minutes of heat treatment. As the temperature increased from 50° to 80°C., the swelling power became less, and except from 60° to 65°C. where the rate of decrease in swelling power was greatest, the alteration was approximately proportional to the temperature. At temperatures from 30° to 50°C. the results indicated that the swelling power was increased. Except for the greater decrease between 60° and 65°C. there was no indication of a coagulation temperature.

Cook (10) showed that the viscosity of an approximately 3% gluten dispersion in 30% urea solution decreased rapidly for the first hour on heat treatment at 70°C., and that thereafter a further but much slower viscosity decrease resulted. Cook also heat treated gliadin of normal moisture content, at 70°C. After treatment the gliadin was dried in vacuum at room temperature and dispersed in 60% alcohol. The viscosity of the solution of gliadin that had been treated 6 hours was not significantly different than that of the control samples. Cook studied the effect of heat treatment at 70°C. on the viscosity of gliadin dispersed in 30% urea solutions. Only in one case was the decrease in viscosity in 1.5 hours over 6% of the initial viscosity, whereas with gluten dispersed in urea solutions the decrease in 1.5 hours was 17.4% of the initial viscosity. These results were taken to indicate that glutenin is the component of gluten more easily altered by heat.

EXPERIMENTAL

I. VISCOSITY OF GLUTEN DISPERSIONS

Material

Source of gluten. The flour, from which the gluten was obtained, was milled in the laboratory from a high-grade sample of Marquis, a variety of hard red spring wheat. The flour was free from bleaching agents and chemical improvers.

Dispersing agents. Merck's C.P. reagents were used.

Methods

Washing of gluten. The flour was weighed out, mixed into a firm dough and allowed to stand for 1 hour in about 4 times its weight of distilled water. It was then washed under a stream of tap water flowing at approximately 75 cc. per minute. When 250 grams of flour were used this washing was continued for 30 minutes under tap water and 10 minutes under distilled water.

With other quantities the time of washing was varied in accordance with the size of sample.

Preparation of dispersions. The average of several determinations showed the wet gluten to contain about 4.3% nitrogen and 67% water. In the preparation of dispersions in urea solutions the desired amount of wet gluten was put in an erlenmeyer flask containing 30% urea solution plus enough solid urea to bring the water in the wet gluten up to 30% urea solution. In all the dispersions, except where otherwise stated, an amount of urea-buffer solution equal to one-fourth the sum of the weights of 30% urea solution, solid urea and wet gluten was added before the wet gluten. This urea-buffer solution was made up of 7 cc. of solution 1 containing 9.078 grams of potassium dihydrogen phosphate (KH_2PO_4) in 200 cc. of 30% urea solution, and 3 cc. of solution 2 containing 9.474 grams of anhydrous disodium hydrogen phosphate (Na_2HPO_4) in 200 cc. of 30% urea. This gave the desired pH of 6.8. It will be observed that these concentrations are 5 times those given by Sorensen. The concentration recommended by Sorensen was obtained on adding 1 part of this buffer to 4 parts of the dispersion.

In preliminary experiments with sodium salicylate, 5, 8, 10, 12, 15 and 20% solutions were used. All of these, except the 5% solution, dispersed the gluten completely, but

the 12% appeared to act most rapidly. The 8% and 10% solutions were almost as rapid as the 12%, the 15% was slightly slower and the 20% solution definitely slower. A concentration of 12% was adopted for further work. Solid reagent was added to correct for the water in the wet gluten. No buffer was used.

In the preparation of dispersions in acid and alkali 0.1 N acetic acid and 0.1 N sodium hydroxide were used. In neither case was any concentrated reagent added to correct for the water in the wet gluten nor was any buffer added.

Immediately after adding the gluten to the dispersing agent, the erlenmeyer flask, the capacity of which was approximately three times the volume of dispersion, was shaken vigorously for about 5 minutes and about 1 minute in every 5 thereafter. During this period the material was kept at 25°C. The approximate times required for dispersion were: in urea, 1 hour; in sodium salicylate, 3 hours; in acetic acid, 1.5 hours and in sodium hydroxide, 2.5 hours.

The dispersions were centrifuged in a Sharples supercentrifuge at a rate of about 50 cc. per minute. This completely removed the starch, but caused little loss of protein. In all cases, except where otherwise stated, the centrifuging of the dispersions in urea, acetic acid and sodium hydroxide was begun when the

dispersions were 3 hours old, i.e. 3 hours after adding the gluten; and that of those in sodium salicylate when 4 hours old.

After centrifuging, the centrifuged dispersions were stored at 25°C.

Analysis of dispersions. The determination of the protein nitrogen content of the dispersions in urea solutions involved the quantitative precipitation of the protein from a known weight of dispersion, complete removal of the urea from this precipitate and the determination of the amount of nitrogen in the precipitate.

The precipitating reagent used was a freshly prepared solution consisting of 50 gm. of tannic acid and 25 cc. of concentrated sulphuric acid per liter of solution. Preliminary experiments showed that trichloracetic acid did not precipitate all the proteins and as this tannic acid reagent proved satisfactory no other protein precipitants were tried.

The procedure was as follows: 40 cc. of the tannic acid reagent was placed in a 100 cc. centrifuge tube and sufficient distilled water added so that, on adding the dispersion, the total volume was about 100 cc. The dispersion was added from a 10 cc. graduated cylinder, the weight of the dispersion being obtained by weighing the cylinder on an analytical balance before and after emptying. Sufficient dispersion was used to give

approximately 50 mgm. of protein nitrogen. After stirring thoroughly, the precipitate was centrifuged out and the liquid decanted. If the dispersion had no foam, little trouble was experienced with particles floating. The precipitate was washed 3 times by adding 5 to 7 cc. of tannic acid reagent, breaking the precipitate up thoroughly with a stirring rod, diluting, while stirring, to about 100 cc. with water and finally centrifuging. After washing, the precipitate was rinsed into a Kjeldahl flask with water and the tube washed first with the concentrated sulphuric acid used for digestion and then with water.

This procedure was tested by analysing dispersions containing a known amount of wet gluten, the nitrogen content of which was determined by the Kjeldahl method. Table I gives the results of these tests. These indicate that the method gives good results, the maximum difference between averages being 0.10 in 8.32 or 1.2%.

In the case of gluten dispersed in sodium salicylate, acetic acid and sodium hydroxide solutions the dispersion was transferred directly to a Kjeldahl flask from a 10 cc. graduated cylinder, the weight of dispersion being obtained by weighing the cylinder on an analytical balance before and after emptying.

The concentrations of gluten are expressed as mgm. of protein nitrogen per gm. of dispersion. This was more easily determined than mgm. per cc. as the dispersions were

too viscous to be pipetted accurately. It also enabled all dilutions to be made by weight rather than by volume as would otherwise have been necessary.

Table I

Recovery of protein nitrogen from dispersions of gluten in 30% urea solutions

Concentration of dispersion % protein (N x 5.7)	Nitrogen content of wet gluten %		Factor used in calculating concentration of dispersion*	Concentration of dispersion mgm. of N per gm.		
				Calculated+	Determined	
		Av.				Av.
2.75	4.27		1.135		4.82	
	4.29				4.83	
	4.27				4.84	
	4.38	4.30		4.88		4.83
4.60	4.26		1.880		8.10	
	4.18				8.00	
	4.23				8.12	
	4.44	4.28		8.05		8.07
4.69	4.32		1.913		8.22	
	4.39				8.21	
	4.35	4.35		8.32	8.22	8.22
5.71	4.66		2.159		10.12	
	4.59				9.94	
	4.70				10.00	
	4.64	4.65		10.04	10.02	10.02
7.59	4.23		3.233		13.33	
	4.26				13.30	
	4.01					
	4.12	4.15		13.42		13.32

* Factor = $\frac{\text{weight gluten x 10}}{\text{weight dispersion}}$

\+ Calculated concentration = nitrogen content of wet gluten (%) x factor.

Salting out tests. In order to obtain some insight into the processes responsible for the viscosity changes observed in experiments to be described later, salting out tests were conducted on gluten dispersed in 30% urea solutions. According to present theories a viscosity decrease must be due to dehydration (16, 17) or to a breaking up of aggregates (28). If dehydration occurred, the amount of protein precipitated by the addition of a limited quantity of salt would increase, but if a breaking up of the aggregates occurred then the amount would decrease. The method was asfollows: a definite quantity of 20% magnesium sulphate (anhydrous) solution was slowly added with shaking to 10 cc. of dispersion. After standing 1 hour at room temperature the precipitate was centrifuged out, washed once with magnesium sulphate urea solution of the same composition as the medium in which precipitation occurred, and then with water. As it was not known whether this precipitate could be washed urea free, the precipitate was redispersed in 30% urea solution and the resulting dispersion analysed for protein nitrogen by the tannic acid precipitation method described before.

Hydrogen-ion concentration. This determination was made at room temperature using a Clark hydrogen electrode, saturated potassium chloride and a Leeds and Northrup Type K potentiometer.

Viscosity determination and standardization of viscometers. All viscosity measurements were made in Standard U-tube viscometers (B.E.S.A.). These were standardized with water and sucrose solutions, using the values given in the International Critical Tables (20) at the temperatures $\pm 0.02^{\circ}$C. stated. All densities were determined at the same temperatures using 25 cc. specific gravity bottles with drilled stoppers. In weighing, corrections were applied for the buoyancy of air.

Table II gives the results of this standardization of viscometers. The greatest deviation occurs with viscometer 11 where the difference between the highest and lowest value of η/dt. is 7 in 900.

All viscosity and density determinations on gluten dispersions were made at $25 \pm 0.02^{\circ}$C.

Table II

Standardization of viscometers

Viscometer	B.E.S.A. range centipoise	Sucrose %	Temperature °C.	Viscosity centipoise	Density gm/cc.	Time of flow sec.	$\eta/dt.$
11	0.9-7.2	0	25	0.8949	0.99704	99.8	.00899
		20		1.710	1.0792	177.3	.00894
		40		5.206	1.1742	492.3	.00901
						Av.	.00898
12	0.9-7.2	0	25			92.6	.00969
		20				164.0	.00966
		40				457.2	.00970
						Av.	.00968
21	5.4-43	40	25	5.206	1.1742	109.1	.0406
		40	20	6.223	1.1765	130.8	.0404
		60	30	34.01	1.2817	654.2	.0406
		60	25	44.02	1.2835	847.3	.0405
						Av.	.0405
22	5.4-43	40	25			116.4	.0381
		40	20			139.2	.0380
		60	30			697.6	.0380
		60	25			904.9	.0379
						Av.	.0380
23	5.4-43	40	25			105.2	.0421
		40	20			125.2	.0422
		60	30			629.1	.0422
		60	25			815.4	.0421
						Av.	.0422

Results

Viscosity of media. The viscosity of buffered 30% urea solution, and of 12% sodium salicylate was 1.22; that of 0.1 N acetic acid, 0.90; and that of 0.1 N sodium

hydroxide, 0.92 centipoises. The International Critical Tables (20) give the relative viscosity of 0.1 N sodium hydroxide at 25°C. as 1.023 which equals 0.92 centipoises. The viscosity of the other solutions is not given.

Effect of storage temperature. Before investigating the effect of any treatment on the viscosity of the dispersions, the viscosity changes which occur during storage had to be studied in order to determine the conditions favoring a reasonably constant viscosity over a period of several days. Preliminary experiments employing 30% urea solutions showed that complete dispersion could not be effected in this reagent at 0°C. At 25°C. the viscosity of the dispersions decreased, at first rapidly and later more slowly. The viscosity of a dispersion stored in ice when only 2 or 3 hours old, continued to decrease, but if the sample were first stored at 25°C. for several hours and then at 0°C., the viscosity remained essentially constant.

To study the effect of storage in urea solution at 0°C. after a preliminary period at 25°C., a dispersion was prepared in the manner previously described except that the dispersion was centrifuged when 1.2 hours old and buffered after centrifuging. The centrifuged dispersion was stored at 25°C. and portions put in ice after 2, 4, 6, 8, 10 and 12 hours. The viscosity change of each portion

was followed for a period of several days. The concentration of gluten in this dispersion was 5.07 mgm. of protein nitrogen per gram of dispersion. This type of experiment was only carried out using this one concentration.

The results are given in Fig. 1. From these it was concluded that an initial storage of 8 hours at 25°C. with subsequent storage at 0°C. would give a reasonably constant viscosity. Consequently this procedure was adopted in the preparation of dispersions of approximately this concentration in urea solutions for heat treatment studies. The results, given in Table III, show that the pH increased slightly during this storage at 0°C., but from data presented later (Table XVIII) it can be seen that these small changes in pH would not affect the viscosity.

Table III

pH of dispersions in 30% urea solution stored at 0°C. after initial storage at 25°C.

Duration of storage at 25°C. hr.	Duration of storage at 0°C.		
	Nil	11 days	19 days
2	--	6.86	6.93
4	6.83	6.89	6.95
6	--	6.89	6.95
8	--	--	--
10	--	6.90	6.96
12	--	6.90	6.95

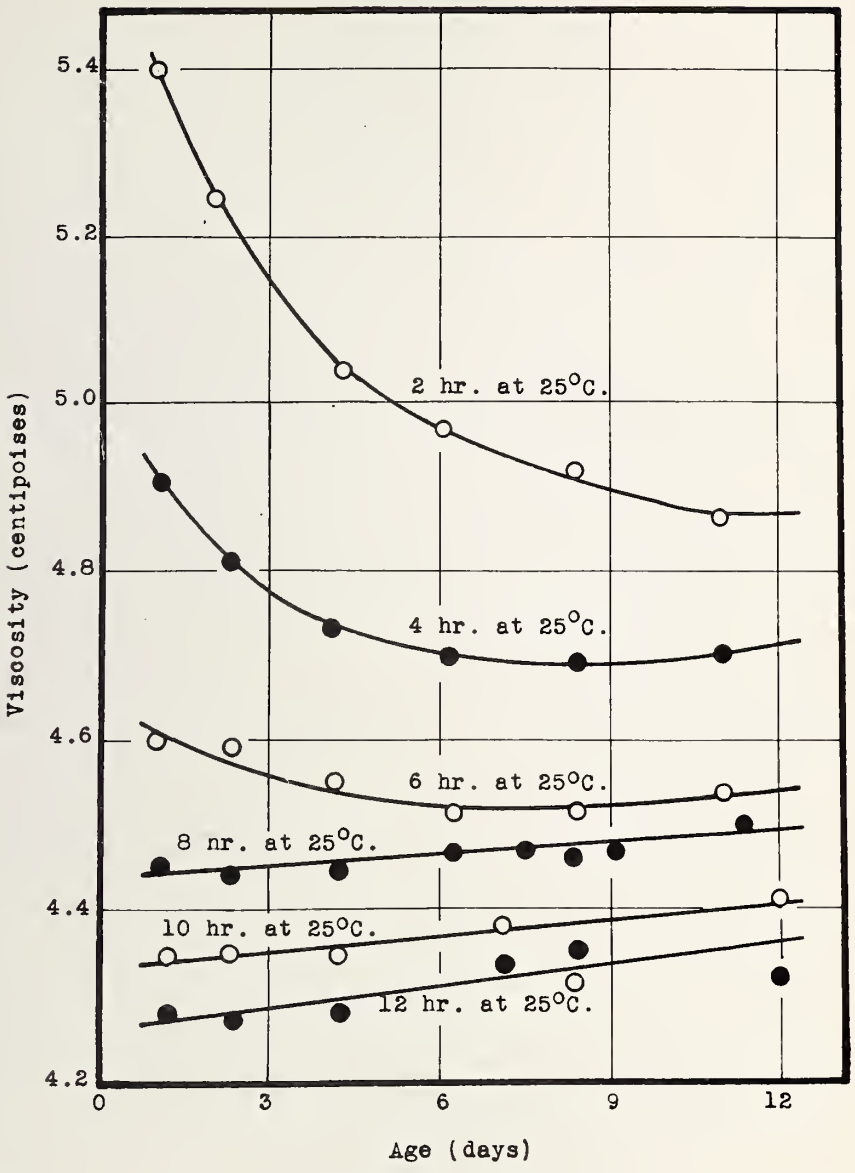

Fig. 1 - Viscosity of gluten in 30% urea solution stored at 0°C. after initial periods at 25°C.

The results in Tables IV and V show the effect of storage at 0°C. on the viscosity of more concentrated dispersions. In these the viscosity showed a definite increase.

Table IV

Viscosity of gluten dispersed in 30% urea solution stored at 0°C. after 4 hours at 25°C.

Conc. = 10.7 mgm. of protein nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise
	4	15.0
1	9.7	16.9
2	1.8	17.4
3	1.5	18.0

Table V

Viscosity of gluten dispersed in 30% urea solution stored at 0°C. after 24 hours at 25°C.

Conc. = 10.0 mgm. of protein nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise
1		10.6
1	7	10.9
2	1.8	11.2
3	1.5	11.3

The results presented in Tables VI, VII and VIII show the effect or storage at 25°C. on the viscosity of dispersions in 30% urea solutions. The dispersion, the viscosity of which is reported in Table VII, was centrifuged when 5 hours old.

Table VI

Viscosity of gluten dispersed in 30% urea solution stored at 25°C.

Conc. = 5.24 mgm. of protein nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	K x 10^3	pH
	4	4.72		
	5	4.57	3.10	
	6	4.43	3.54	
	7	4.34	2.76	
	8	4.25	3.30	6.80
	10	4.12	3.18	
	12	4.05	2.39	
	14	4.01	1.76	
	20	3.90	2.89	
1		3.87	2.89	
1	4	3.85		
1	10	3.84		
1	22	3.83		
2	12	3.81		
3		3.80		
4		3.79		
5		3.77		
6		3.76		
7	12	3.75		
9		3.72		
10		3.73		6.98

Table VII

Viscosity of gluten dispersed in 30% urea solution stored at 25°C.

Conc. = 9.48 mgm. of protein nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	K x 10^3
	6.3	12.4	
	7	12.2	3.68
	8	11.9	4.79
	10	11.6	3.38
	12	11.3	
	22	11.0	
1	23	11.1	

Table VIII

Viscosity of gluten dispersed in 30% urea solution stored at 25°C.

Conc. = 10.7 mgm. of protein nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	K x 10^3
	4	15.0	
	5	14.4	4.37
	6	14.0	3.72
	7	13.7	3.45
	8	13.5	2.77
	9	13.3	3.34
	10	13.2	
	12.1	13.0	
	16.2	12.7	
	20	12.6	
	23	12.6	
1		12.6	
1	6.5	12.4	
2	2.5	12.6	
2	8.5	12.8	
2	23.5	12.9	

The results in Tables IX and X show the effect of storage at 25°C. on the viscosity of dispersions in 12% sodium salicylate solutions.

Table IX

Viscosity of gluten dispersed in 12% sodium salicylate stored at 25°C.

Conc. = 5.08 mgm. of nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	pH
	5	4.20	
	10	4.08	
	22	4.02	
1	10	4.02	
1	22	4.05	
2	8	4.08	
2	22	4.06	6.90
3	22	4.08	
5	3	4.08	
6	5	4.08	

Table X

Viscosity of gluten dispersed in 12% sodium salicylate stored at 25°C.

Conc. = 10.7 mgm. of nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	pH
	5	16.8	
	10	17.5	
	22	18.1	
1	10	18.8	
1	22	19.7	
2	8	20.2	
2	22	20.6	
3	22	21.0	
5	3	21.3	
6	5	21.8	
8		22.0	6.87

The results given in Tables XI, XII and XIII show the effect of storage at 25°C. on the viscosity of dispersions in 0.1 N acetic acid.

Table XI

Viscosity of gluten dispersed in 0.1 N acetic acid stored at 25°C.

Conc. = 5.17 mgm. of nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	K x 10^3	pH
	4	3.68		
	5	3.51	1.47	
	6.2	3.37	1.09	
	8	3.13	1.41	
	10	2.94	1.16	
	21	2.32	1.01	
1		2.24		
1	4	2.15		
1	12	2.00		
1	22	1.89		
2	4	1.87		
2	10	1.81		
2	22	1.75		3.64
3	5	1.72		
3	22	1.67		

Table XII

Viscosity of gluten dispersed in 0.1 N acetic acid stored at 25°C.

Conc. = 5.46 mgm. of nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	K x 10^3	pH
	4.5	4.13		
	5.5	3.99	0.99	
	6	3.90	1.34	
	7.5	3.72	0.96	
	10	3.42	1.08	
	12	3.20	1.16	
	22	2.47	1.13	
1		2.40		
1	12	2.12		
2	6	1.92		
2	12	1.88		
3		1.82		
4		1.72		3.74
5		1.67		
6		1.63		

Table XIII

Viscosity of gluten dispersed in 0.1 N acetic acid stored at 25°C.

Conc. = 10.3 mgm. of nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	K x 10^3	pH
	4.5	9.15		
	5.5	8.70	1.30	
	6	8.45	1.53	
	7.5	7.84	1.36	
	10	6.99	1.34	
	12	6.61		
	22	5.04		
1		4.86		
1	12	4.23		
1	13.2	4.20		
2	6	3.77		
2	12	3.64		
3		3.46		
4		3.16		4.02
5		3.00		
6		2.89		

The results given in Tables XIV and XV show the effect of storage at 25°C. on the viscosity of dispersions in 0.1 N sodium hydroxide.

Table XIV

Viscosity of gluten dispersed in 0.1 N sodium hydroxide stored at 25°C.

Conc. = 5.46 mgm. of nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	pH
	3.8	1.92	
	5.2	1.90	
	10	1.86	
	22	1.84	12.6
1	10	1.82	
2		1.79	
3	3	1.74	
4	1	1.73	

Table XV

Viscosity of gluten dispersed in 0.1 N sodium hydroxide stored at 25°C.

Conc. = 10.2 mgm. of nitrogen per gm. of dispersion.

Age day	Age hr.	Viscosity centipoise	pH
	4.5	3.93	
	5.5	3.92	
	10.2	3.92	
	13.5	3.92	
	22	3.86	12.2
1	10	3.78	
2		3.70	
3	3	3.59	
4	1	3.60	

The results given in Tables VI to XV are presented graphically, those of dilute dispersions (5.08 to 5.46 mgm. of protein nitrogen per gram of dispersion) in Fig. 2, and those of concentrated dispersions (9.48 to 10.7 mgm. of protein nitrogen per gram of dispersion) in Fig. 3.

The nature of the viscosity decrease. Salting out tests were made on a dispersion in buffered 30% urea solution containing 4.52 mgm. of protein nitrogen per gram of dispersion. The results given in Table XVI show the amount of nitrogen precipitated from 10 cc. portions after 4 hours and again after 46 hours of storage at 25°C. During this period the viscosity of the dispersion decreased from 4.94 to 3.99 centipoises and the pH increased from 6.76 to 6.83.

Table XVI

Effect of storage on the subsequent precipitation of protein

20% $MgSO_4$ used cc.	Mgm. nitrogen precipitated	
	When dispersion 4 hr. old	When dispersion 46 hr. old
2.75	12.2	6.1
2.75	14.6	7.4
3.00	22.1	20.7
3.00	24.0	20.6
3.50	34.4	30.8
3.50	31.2	30.1

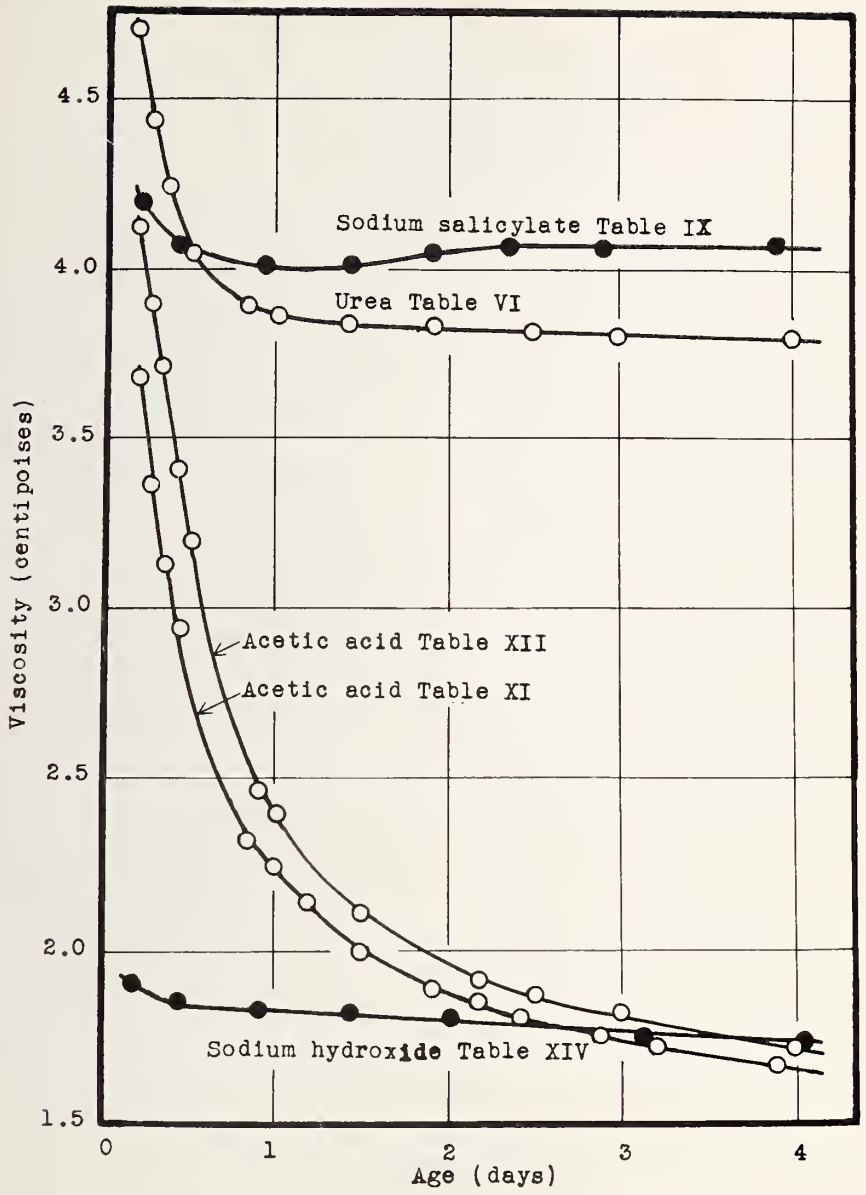

Fig. 2 - Viscosity of dilute gluten dispersion stored at 25°C.

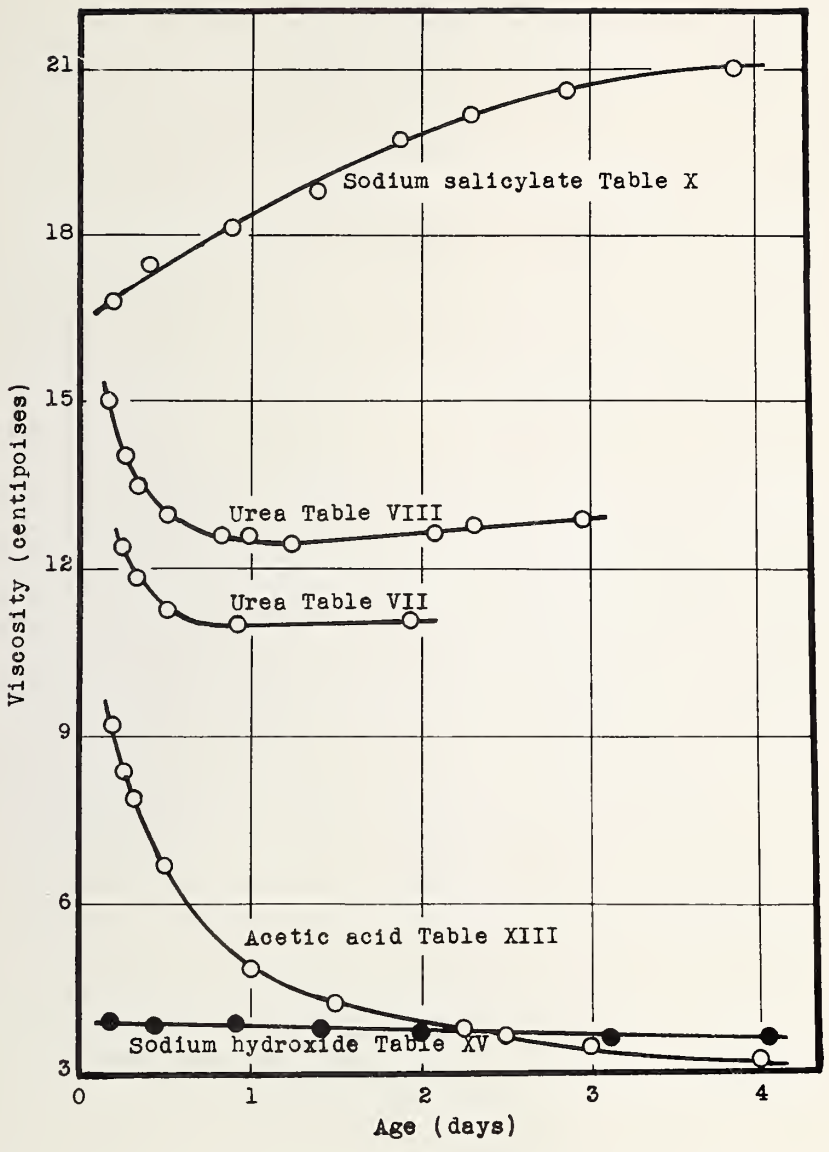

Fig. 3 - Viscosity of concentrated gluten dispersions stored at 25°C.

These results show that the amount of protein precipitated, by the method adopted, decreased with storage at 25°C. This, along with the decrease in viscosity, indicates that the size of the particles decreased due, probably, to a break down of the aggregates.

Dispersions of gluten in acetic acid were analysed using the tannic acid precipitation method. The recovery from a dispersion that was 5 hours old was 98.6% and that from a dispersion 3 weeks old was 88.2%. The decrease indicates that hydrolysis had occurred.

A precipitate formed in the dispersions in sodium hydroxide after they had stood at room temperature for about 2 weeks. The odor of ammonia was also evident at this time.

The order and velocity of reaction. As different concentrations of gluten were used, it was possible to calculate the order of the reaction responsible for the viscosity change which occurred during storage at 25°C. A summary of the values used in calculating the order of reaction in dispersions in urea and acetic acid solutions is given in Table XVII.

The constancy of the values of $t_m - t_1$ in each dispersing reagent indicates that the reaction is unimolecular. This conclusion is in accordance with that of Lewis (23, 24) who studied the heat denaturation of oxyhemoglobin and of egg albumin.

Table XVII

Values used in determining the order of reaction

Table from which values are obtained	Concentration of gluten N mgm/gm.	η_1 centi-poise	η_∞ centi-poise	η_m centi-poise	$t_m - t_1$ min.
Dispersions in urea solutions					
VI	5.24	4.72	3.84	4.28	210
VII	9.48	12.4	11.0	11.7	190
VIII	10.7	15.0	12.4	13.7	180
Dispersions in acetic acid solutions					
XI	5.17	3.68	1.67	2.67	540
XII	5.46	4.13	1.72	2.92	570
XIII	10.3	9.15	3.16	6.16	570

$$\eta_m = \frac{\eta_1 + \eta_\infty}{2}$$

$t_m = t$ when $\eta = \eta_m$

The unimolecular reaction constants for these dispersions were calculated using the equation $K = \frac{1}{t_2 - t_1} \ln \frac{\eta_1 - \eta_\infty}{\eta_2 - \eta_\infty}$ where t = time in minutes. The values of η_∞ used are given in Table XVII. As there is no evidence that the viscosity ever becomes absolutely constant, it was necessary to select arbitrary values for η_∞.

From Table VI and Fig. 2 it can be seen that the viscosity of the dilute gluten dispersion in 30% urea solution is essentially constant after 1 day 10 hours. Therefore, the viscosity at this time was selected as η_∞. For the other dispersions in urea solutions the lowest

viscosities recorded in Tables VII and VIII were selected as the values for η_∞ . Apparently the viscosities of the gluten dispersions in acetic acid continued to decrease indefinitely. Since the viscosity of the first dispersion prepared in acetic acid solution was last determined when the dispersion was about 4 days old, and since all these dispersions behaved in a similar manner, the viscosity at this time was selected as η_∞ in each case.

The reaction constants were not calculated for dispersions in sodium salicylate and sodium hydroxide. In these cases the viscosity changes observed were slight and consequently the experimental error would play a large part in determining the value of the reaction constants.

In dealing with the viscosity behavior of gluten in different solvents certain features are evident. The magnitude of the viscosity and its change on storage depends on the dispersing agent used. The viscosity of the dispersions in the neutral solvents was, in all cases, higher than that of the dispersions in acids and alkalis. Alkalis appear to act very rapidly on the dispersed gluten as shown by the low level of the viscosity when first determined. Acids appear to affect the same viscosity changes as alkalis but at a slower rate.

Effect of hydrogen-ion concentration on viscosity. This study was undertaken to determine the limits of pH

within which the viscosity could be considered constant. This was necessary as it was known that urea solutions tend to become alkaline when heat treated, or when stored.

An unbuffered dispersion containing approximately 6.25 mgm. of protein nitrogen per gram of dispersion was prepared in 30% urea solution. Buffer solutions 1 and 2 (page 16) were mixed in the proportion shown in Table XVIII. Beginning when the dispersion was 6.5 hours old, samples were prepared by adding 10 gm. of the buffer solutions to 40 gm. of the dispersion. The preparation of the entire series required about 45 minutes. By mixing these proportions the gluten was diluted to approximately 5 mgm. of protein nitrogen per gram of dispersion and the buffer to the strength recommended by Sorensen. The samples were prepared in the order 6, 7, 5, 8, 4, 9, 3, 10, 2, 11, 1, 12 and then 5A and 6A. This order was adopted as it was known that the viscosity of the stock dispersion would be changing as shown in Table VI, and it was hoped that this procedure would show the effect, if any, of age at time of buffering. All the samples were put in ice when the dispersion was 8 hours old.

The viscosities and pH values were determined 1 day later and again 4 days later, but in no case did there appear to be any significant change. Portions of samples 1, 6 and 12 were analysed for protein nitrogen. As

the dispersion medium had a different composition for each hydrogen-ion concentration, the viscosities of the media were determined. These were essentially the same, varying only from 1.22 centipoises at pH 6.1 to 1.25 centipoises at pH 8.0.

The results obtained 4 days after buffering are given in Table XVIII, and they indicate that over the range from 6.1 to 8.0, pH has no effect on viscosity. The protein nitrogen content of the samples that were analysed was essentially the same. That the viscosity is independent of pH is in agreement with the results of Loughlin and Lewis (27) who found that the viscosity of egg albumin and oxyhemoglobin was independent of pH.

Table XVIII

Effect of hydrogen-ion concentration on the viscosity of a gluten dispersion in 30% urea solution

Sample	Composition of buffer		Viscosity	pH	Concentration (duplicate)	
	Na_2HPO_4 solution cc.	KH_2PO_4 solution cc.	centipoise		mgm/gm.	
1	0.25	9.75	4.11	6.12	5.02	5.02
2	0.50	9.50	4.10	6.20		
3	1	9	4.10	6.37		
4	2	8	4.10	6.61		
5	3	7	4.10	6.80		
5A	3	7	4.10	--		
6	4	6	4.10	6.99	5.00	5.01
6A	4	6	4.09	--		
7	5	5	4.10	7.12		
8	6	4	4.12	7.22		
9	7	3	4.11	7.39		
10	8	2	4.09	7.65		
11	9	1	4.09	7.95		
12	9.5	0.5	4.08	8.05	5.01	5.02

II. EFFECT OF HEAT TREATMENT ON THE VISCOSITY OF GLUTEN DISPERSIONS

Material

The materials were the same as those used in the studies reported in Part I.

Methods

The methods described in Part I were employed in the studies reported in this part. For heat treatment studies, dispersions of gluten concentrations slightly higher than those desired were prepared, analysed and then diluted to the desired concentration. The dispersions, containing 5.00 mgm. of protein nitrogen per gram of dispersion, in 30% urea solutions were stored at 0°C. after 8 hours at 25°C. All other dispersions were stored at 25°C. prior to heat treatment.

Two baths were used for heat treating purposes. These were well insulated, equipped with stirrers and fitted with wooden lids with holes, through which narrow test tubes containing the dispersion to be treated were

placed. One bath contained mineral seal oil, the temperature of which was controlled by means of a DeKhotinsky bimetallic thermoregulator. The other bath contained water, the temperature of which was controlled by adjusting a tubular resistance by hand. This method proved satisfactory, but when the test tubes of dispersion were first placed in the bath the resistance had to be lowered and the temperature watched closely for a few minutes.

The treatments were carried out at temperatures $\pm 0.5^{\circ}$C. recorded. The length of the period from the time the sample was put in the bath until it was removed was taken as the duration of heat treatment. The hot sample was placed in the viscometer and cooled in the bath at 25°C.

Results

In this section dispersions containing 5 mgm. of protein nitrogen per gram of dispersion are referred to as "dilute dispersions" and those containing 10 mgm. of protein nitrogen per gram of dispersion as "concentrated dispersions".

The viscosities of the media were the same as those reported in Part I.

Heat decomposition of 30% urea solutions. In order to study the heat decomposition of buffered urea solutions, 50 cc. portions were treated at 80°C. in sealed tubes. After

treatment, the solutions were cooled, transferred to flasks in ice water, saturated with magnesium oxide and aerated. The ammonia given off was collected in standard acid and the amount determined hourly. The results, given in Table XIX, show that the urea decomposed on heating. However, after treatment for 4 hours at 80°C. only 30 mgm. of nitrogen were collected and as 50 cc. of 30% urea solution contains 7000 mgm. of nitrogen it is obvious that the decomposition did not affect the urea concentration significantly.

Table XIX

Effect of heat treatment at 80°C. on the decomposition of urea solutions

Duration of treatment hr.	Nitrogen collected during aeration (mgm.)						
	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.	6th hr.	7th hr.
0	0.10						
1	5.5	0.8	0.10				
2	13.2	1.1	0.8				
4	23.6	4.4	1.5	0.4	0.20	0.14	0.10

Heat treatment of dispersions in 30% urea solutions.

Three dilute dispersions were prepared and portions heat treated. The results are given in Tables XX to XXV. In each case the dispersion used, and the age of the dispersion when treatment was begun are recorded. The results obtained during the initial stages of heat treatment are shown in Fig. 4.

Dispersion II was treated at 80°C. when 8 hours old, and again when 4 days old. The results (Table XXIII) are essentially the same and indicate that storage at 0°C. did not affect the viscosity behavior at 80°C.

At 80°, 90° and 100°C., (Tables XXIII, XXIV and XXV) the viscosity increased when the pH increased beyond 8.0. It is impossible, without further study, to say whether or not the increase was due to the effect of hydrogen-ion concentration. At 100°C. the dispersion turned dark and a precipitate showed after 5.5 hours of treatment.

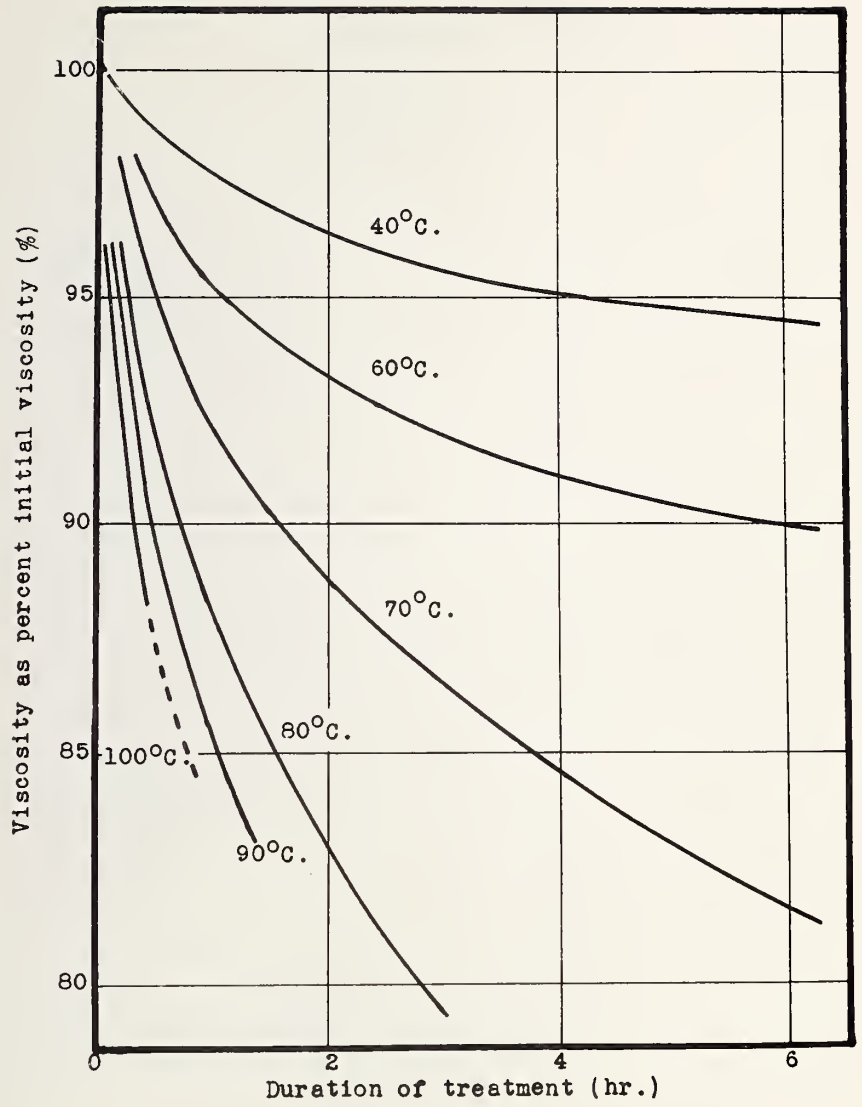

Fig. 4 - Effect of heat treatment on the viscosity of dilute dispersions in 30% urea solutions.

Table XX

Effect of heat treatment at 40°C. on the viscosity of dilute dispersions in 30% urea solutions

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
		Dispersion I, 2 days old		
	0	4.14	100.0	6.82
	5	4.13	99.8	
	30	4.13	99.8	
1		4.06	98.1	6.85
1	45	4.01	96.6	
2	30	4.00	96.5	6.84
3	15	3.98	96.1	
4		3.97	95.9	
5		3.95	95.4	
6		3.94	95.2	6.87
7	30	3.98	96.1	
8	15	3.96	95.7	
9		3.96	95.7	6.88
		Dispersion II, 1 day old		
	0	4.11	100.0	6.80
	5	4.08	99.3	
	30	4.05	98.5	6.81
1		4.01	97.6	6.81
1	30	4.00	97.3	6.82
2		4.00	97.3	6.82
3		3.97	96.6	6.82
4		3.94	95.9	6.85
5		3.92	95.4	6.85
6		3.91	95.1	6.86
7		3.89	94.6	6.88
11		3.89	94.6	6.87
		Dispersion III, 1 day old		
	0	4.03	100.0	6.80
	5	4.02	99.8	
	30	3.97	98.5	
1		3.92	97.3	
2		3.85	95.5	6.82
3		3.80	94.3	
4		3.81	94.5	6.84
5		3.75	93.1	
6		3.73	92.6	6.83
7		3.70	91.8	
8		3.71	92.1	

Table XXI

Effect of heat treatment at 60°C. on the viscosity of dilute dispersions in 30% urea solutions

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
		Dispersion I, 2 days old		
	0	4.13	100.0	6.82
	8	4.14	100.2	
	30	4.01	97.1	
1		3.93	95.2	6.86
1	30	3.91	94.7	
2		3.86	93.5	6.90
2	30	3.83	92.7	
3		3.80	92.0	6.96
3	45	3.75	90.8	
4	30	3.76	91.0	7.09
5	15	3.72	90.1	
6	15	3.71	89.8	7.21
7	30	3.65	88.4	
8	30	3.59	86.9	
9		3.62	87.7	7.35
		Dispersion II, 1 day old		
	0	4.12	100.0	6.80
	10	4.09	99.3	
	30	4.00	97.1	6.82
1		3.89	94.4	6.83
1	30	3.84	93.2	6.83
2		3.85	93.4	6.86
3		3.79	92.0	6.92
4		3.76	91.3	7.02
5		3.72	90.3	7.08
6		3.72	90.3	7.14
7		3.71	90.0	7.17
8		3.69	89.6	7.26
9		3.64	88.3	7.30
12		3.56	86.4	7.42

Table XXII

Effect of heat treatment at 70°C. on the viscosity of dilute dispersions in 30% urea solutions

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
		Dispersion II, 3 days old		
	0	4.12	100.0	6.85
	10	4.11	99.8	
	30	3.94	95.6	6.85
1		3.82	92.7	6.91
1	30	3.79	92.0	7.03
2		3.71	90.0	7.12
2	30	3.68	89.3	7.21
3		3.61	87.6	7.31
4		3.52	85.4	7.49
5		3.44	83.5	7.68
6		3.36	81.6	7.88
7		3.30	80.1	7.90
8	30	3.20	77.7	8.06
10		3.14	76.2	8.26
11		3.10	75.2	
13	20	3.09	75.0	8.61
		Dispersion III, 2 days old		
	0	4.03	100.0	6.80
	10	3.94	97.8	
	30	3.76	93.3	
1		3.66	90.8	6.91
2		3.54	87.8	7.10
3		3.46	85.9	7.28
4		3.39	84.1	7.44
5		3.33	82.6	7.55
6		3.28	81.4	7.68
7		3.22	79.9	7.77
8		3.18	78.9	7.99

Table XXIII

Effect of heat treatment at 80°C. on the viscosity of dilute dispersions in 30% urea solutions

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
		Dispersion I, 1 day old		
	0	4.12	100.0	6.81
	5	3.99	96.8	
	23	3.75	91.0	
	38	3.74	90.8	7.27
1	36	3.48	84.5	7.63
2	13	3.35	81.3	
3	5	3.24	78.6	8.09
4		3.44	83.5	8.54
5		3.77	91.5	
6		3.81	92.5	8.74
7		3.45	83.7	8.94
8		3.26	79.1	8.93
		Dispersion I, 3 days old		
	0	4.14	100.0	6.83
	10	3.98	96.1	
	30	3.80	91.8	7.00
1		3.62	87.4	7.20
1	30	3.50	84.5	
2		3.40	82.1	7.62
2	30	3.32	80.2	
3		3.27	79.0	7.98
3	30	3.23	78.0	8.30
4		3.32	80.2	8.32
4	30	3.34	80.7	8.50
5		3.39	81.9	8.77

Table XXIII (Continued)

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
		Dispersion II, 8 hours old		
	0	4.05	100.0	6.79
	10	3.87	95.5	
	30	3.68	90.9	6.84
1		3.59	88.6	7.10
1	30	3.45	85.2	7.41
2		3.43	84.7	7.49
2	30	3.40	84.0	7.80
3		3.27	80.7	7.93
3	30	3.32	82.0	8.14
4		3.36	83.0	8.30
4	30	3.63	89.6	8.50
5		3.80	93.8	8.59
5	30	3.80	93.8	8.68
6		3.77	93.1	8.76
6	30	3.71	91.6	8.88
		Dispersion II, 4 days old		
	0	4.11	100.0	6.82
	10	3.97	96.6	6.85
	30	3.81	92.7	
1	2	3.66	89.1	7.20
1	33	3.54	86.1	
2		3.44	83.7	7.62
2	30	3.40	82.7	7.84
3		3.31	80.5	8.01
3	30	3.30	80.3	8.25
4		3.43	83.5	8.55
4	30	3.64	88.6	8.60
5		3.73	90.8	8.56
5	30	3.75	91.2	8.71
6		3.76	91.5	
6	30	3.67	89.3	
7		--	--	8.85

Table XXIV

Effect of heat treatment at 90°C. on the viscosity of dilute dispersions in 30% urea solutions

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
		Dispersion II, 2 days old		
	0	4.12	100.0	6.79
	10	3.86	93.7	
	30	3.67	89.1	7.29
	55	3.53	85.7	7.74
1	10	3.53	85.7	7.94
1	30	3.94	95.6	8.23
1	51	4.05	98.3	8.50
2	10	3.93	95.4	8.67
2	35	3.75	91.0	8.82
3		3.59	87.1	8.91
3	30	3.51	85.2	9.04
4		3.38	82.0	9.14
4	30	3.37	81.8	
5		3.36	81.6	9.16
5	30	3.36	81.6	
6		3.33	80.8	9.26

Table XXV

Effect of heat treatment at 100°C. on the viscosity of dilute dispersions in 30% urea solutions

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
		Dispersion I, 3 days old		
	0	4.14	100.0	6.83
	10	3.82	92.3	
	30	3.62	87.4	7.97
1		3.70	89.4	8.74
1	30	3.32	80.2	9.02
2		3.07	74.2	9.15
2	30	2.91	70.3	9.25
3		2.81	67.9	9.26
4	30	2.93	70.8	
5		2.86	69.0	9.35
6		3.13	75.6	9.45
		Dispersion II, 2 days old		
	0	4.11	100.0	
	10	3.81	92.7	
	20	3.73	90.8	7.45
	30	4.22	102.7	7.85
	45	4.05	98.5	8.39
1		3.72	90.5	8.71
1	20	3.46	84.2	
1	40	3.35	81.5	9.17
2		3.19	77.6	
2	30	3.09	75.2	9.39
3		3.04	74.0	
3	30	3.02	73.5	9.39
4		3.13	76.2	
5		3.24	78.8	9.35

The results given in Table XXVI show the effect of heat treatment at 60° and 80°C. on the viscosity of concentrated dispersions. At 60°C. the viscosity decreased slightly and at 80°C. it increased considerably.

Table XXVI

Effect of heat treatment on the viscosity of concentrated dispersions in 30% urea solutions

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
Temperature = 60°C. Dispersion 1 day old				
	0	10.6	100.0	
	10	10.8	101.9	
	30	10.7	100.9	6.82
1		10.6	100.0	6.81
2		10.5	99.1	6.86
3		10.4	98.1	6.93
4		10.4	98.1	6.95
Temperature = 80°C. Dispersion 1 day old				
	0	10.6	100.0	6.79
	10	10.8	101.9	
	30	11.0	103.8	6.89
1		11.6	109.4	7.06
1	30	12.0	113.2	7.27
2		12.8	120.7	7.45
2	45	14.5	136.8	7.72

The results in Table XXVII show the effect of storage at 0°C. on the viscosity of dispersions which had been heat treated. The viscosity remained essentially

constant in all cases showing that this internal condition of the heat treated material was essentially stable.

Table XXVII

Viscosity of dispersions in 30% urea solutions stored at 25°C. after heat treatment

Treatment	Concentration of protein nitrogen mgm/gm.	Duration of storage at 0°C. day	hr.	Viscosity centipoise	pH
9.5 hr. at 40°C.	4.52		0	3.91	6.88
		1		3.92	
		3		3.94	
		10		3.90	6.87
2 hr. at 70°C.	4.52		0	3.58	7.11
		1		3.55	
		3		3.56	
		10		3.55	7.10
4 hr. at 70°C.	5.00		0	3.38	7.44
			10	3.37	
		1		3.39	
		2		3.39	
		8		3.39	7.49
8 hr. at 70°C.	5.00		0	3.17	7.99
			5	3.13	
		1		3.10	
		2		3.11	
		8		3.13	7.89
1 hr. at 80°C.	4.52		0	3.51	7.20
		1		3.49	
		3		3.49	
		10		3.50	7.35

Heat treatment of dispersions in 12% sodium salicylate solutions. The results given in Table XXVIII show the effect of heat treatment at 60° and 80°C. on the viscosity

of dilute dispersions. At 60°C. the viscosity increased and at 80°C. it decreased. During the same period the viscosity of a portion stored at 25°C. increased from 3.94 to 3.98 centipoises.

Table XXVIII

Effect of heat treatment on the viscosity of dilute dispersions in 12% sodium salicylate solutions

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
Temperature 60°C. Dispersion 3 days old				
	0	3.94	100.0	6.90
	30	4.15	105.3	
1		4.14	105.1	
1	30	4.16	105.6	
2		4.20	106.6	
3		4.24	107.6	
4		4.24	107.6	6.86
5	30	4.30	109.1	
10	30	4.30	109.1	
12		4.32	109.6	6.89
Temperature 80°C. Dispersion 3 days old				
	0	3.94	100.0	6.90
	30	4.04	102.5	
1		4.00	101.5	
1	30	3.98	101.0	
2		3.91	99.2	
3		3.87	98.2	
4		3.83	97.2	6.89
5	30	3.76	95.4	
10	30	3.58	90.9	
12		3.55	90.1	6.90

The results given in Table XXIX show the effect of heat treatment at 60° and 80°C. on the viscosity of concentrated dispersions. In order to determine whether evaporation occurred during the removal of samples to the extent that it increased the viscosity, portions of these dispersions were treated in sealed tubes. These were removed at the end of the 12-hour period and cooled to 25°C. before opening. The results show that at 60°C. evaporation affected the viscosity; the viscosity of the material treated in the sealed tube being 22.2, and that of the other 22.7 centipoises. Unfortunately, due to an accident, a direct comparison of the results obtained from treatment at 80°C. is impossible. At 60°C. the viscosity increased and at 80°C. it decreased. During the same period the viscosity of a portion stored at 25°C. increased from 16.8 to 17.1 centipoises.

Table XXIX

Effect of heat treatment on the viscosity of concentrated dispersions in 12% sodium salicylate solutions

Duration of treatment hr.	min.	Viscosity centipoise	Viscosity as % of initial viscosity %	pH
Temperature 60°C. Dispersion 9 days old				
	0	16.8	100.0	6.87
	30	18.5	110.1	
1		19.1	113.7	
1	30	19.5	116.1	
2		19.8	117.9	
4		20.6	122.6	6.89
6		21.1	125.6	
8	30	22.0	131.0	
12		22.7	135.1	6.86
12 (sealed tube)		22.2	132.1	
Temperature 80°C. Dispersion 9 days old				
	0	16.8	100.0	6.87
	30	17.8	106.0	
1		17.3	103.8	
1	30	17.3	103.0	
2		16.9	100.6	
4		15.9	94.6	6.88
6		15.0	89.3	
8	30	14.7	87.5	
12 (sealed tube)		12.9	76.8	6.86

Heat treatment of dispersions in 0.1 N acetic acid. The results given in Tables XXX and XXXI show the effect of heat treatment at 60° and 80°C. on the viscosity of dilute and concentrated dispersions, respectively. In

all cases the viscosity decreased, the change being greatest at the higher temperature. During the same period the viscosity of a portion of the dilute dispersion stored at 25°C. decreased from 1.63 to 1.62 centipoises and that of a portion of the concentrated dispersion decreased from 2.99 to 2.95 centipoises in 6 hours and to 2.92 centipoises in 11 hours.

Table XXX

Effect of heat treatment on the viscosity of dilute dispersions in 0.1 N acetic acid

Duration of treatment hr.	min.	Viscosity centipoise	Viscosity as % of initial viscosity %	pH
Temperature 60°C. Dispersion 4 days old				
0		1.63	100.0	3.74
1		1.63	100.0	
2		1.62	99.4	
4		1.61	98.8	3.77
6		1.61	98.8	
9		1.60	98.2	
12		1.60	98.2	3.75
Temperature 80°C. Dispersion 4 days old				
	0	1.63	100.0	3.74
	30	1.63	100.0	
1		1.61	98.8	
2		1.58	96.9	
4		1.58	96.9	3.81
6		1.56	95.7	
9		1.55	95.1	
12		1.54	94.5	3.79

Table XXXI

Effect of heat treatment on the viscosity of concentrated dispersions in 0.1 N acetic acid

Duration of treatment hr.	min.	Viscosity centipoise	Viscosity as % of initial viscosity %	pH
Temperature 60°C. Dispersion 4 days old				
0		2.99	100.0	4.02
1		2.99	100.0	
2		2.97	99.3	
4		2.93	98.0	4.06
6		2.92	97.7	
9		2.89	96.7	
12		2.87	96.0	4.05
Temperature 80°C. Dispersion 4 days old				
	0	2.99	100.0	4.02
	30	3.04	101.7	
1		2.99	100.0	
2		2.95	98.7	
4		2.93	98.0	4.05
6		2.86	95.7	
11		2.82	94.3	
12		2.81	94.0	4.06

Heat treatment of dispersions in 0.1 N sodium hydroxide. The results given in Tables XXXII and XXXIII show the effect of heat treatment at 60°C. and 80°C. on the viscosity of dilute and concentrated dispersions, respectively. In all cases the viscosity decreased, the change being greatest at the higher temperature. During the same period the viscosity of a portion of the

dilute dispersion decreased from 1.71 to 1.69 centipoises and that of a portion of the concentrated dispersion from 3.74 to 3.66 centipoises.

Table XXXII

Effect of heat treatment on the viscosity of dilute dispersions in 0.1 N sodium hydroxide

Duration of treatment		Viscosity	Viscosity as % of initial viscosity	pH
hr.	min.	centipoise	%	
Temperature 60°C. Dispersion 1 day old				
0		1.71	100.0	12.6
1		1.61	94.1	12.5
2		1.56	91.2	
3		1.52	88.9	
4		1.49	87.1	12.4
6		1.46	85.4	12.3
9		1.43	83.6	12.1
12		1.41	82.4	12.2
Temperature 80°C. Dispersion 1 day old				
	0	1.71	100.0	12.6
	30	1.52	88.9	
1		1.42	83.0	
2		1.37	80.1	12.0
3		1.34	78.4	
4		1.32	77.2	
6	30	1.30	76.0	11.8
8		1.30	76.0	11.6
10		1.29	75.4	
12		1.28	74.9	11.3

Table XXXIII

Effect of heat treatment on the viscosity of concentrated dispersions in 0.1 N sodium hydroxide

Duration of treatment hr.	min.	Viscosity centipoise	Viscosity as % of initial viscosity %	pH
Temperature 60°C. Dispersion 1 day old				
0		3.74	100.0	12.2
1		3.43	91.7	12.0
2		3.27	87.4	
3		3.14	84.0	
4		3.06	81.8	11.7
6		2.92	78.1	11.5
9		2.80	74.9	11.2
12		2.68	71.7	11.2
Temperature 80°C. Dispersion 1 day old				
	0	3.74	100.0	12.2
	30	3.11	83.2	11.8
1		2.81	75.1	11.6
2		2.51	67.1	11.2
3		2.37	63.4	
4		2.28	61.0	10.7
5		2.22	59.4	
6		2.17	58.0	10.5
8		2.13	57.0	
10		2.11	56.4	10.2
12		2.11	56.4	10.2

The effect of heat treatment at 60° and 80°C. on the viscosity of dilute dispersions is shown in Fig. 5, and on the viscosity of concentrated dispersions in Fig. 6. It is evident that the magnitude and direction of the viscosity change depends on the dispersing agent, the concentration of gluten and the temperature of heat treatment.

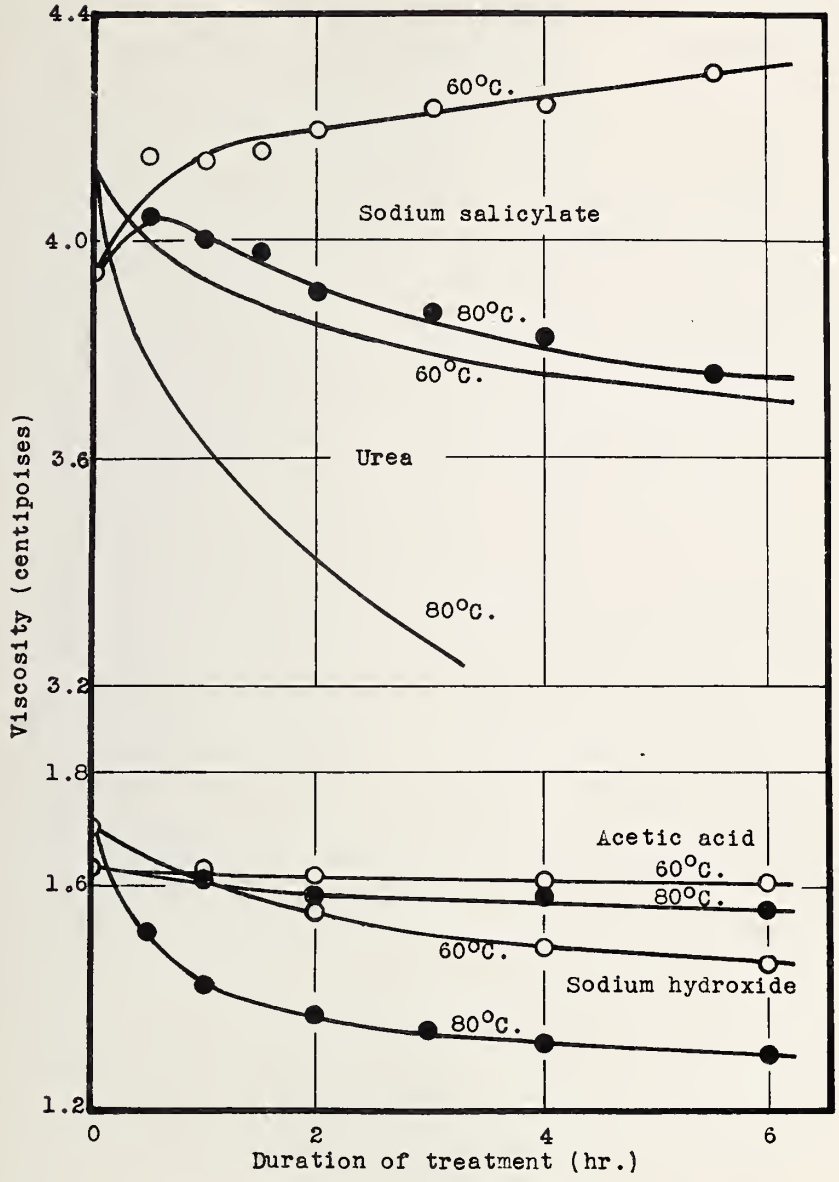

Fig. 5 - Effect of heat treatment on the viscosity of dilute dispersions.

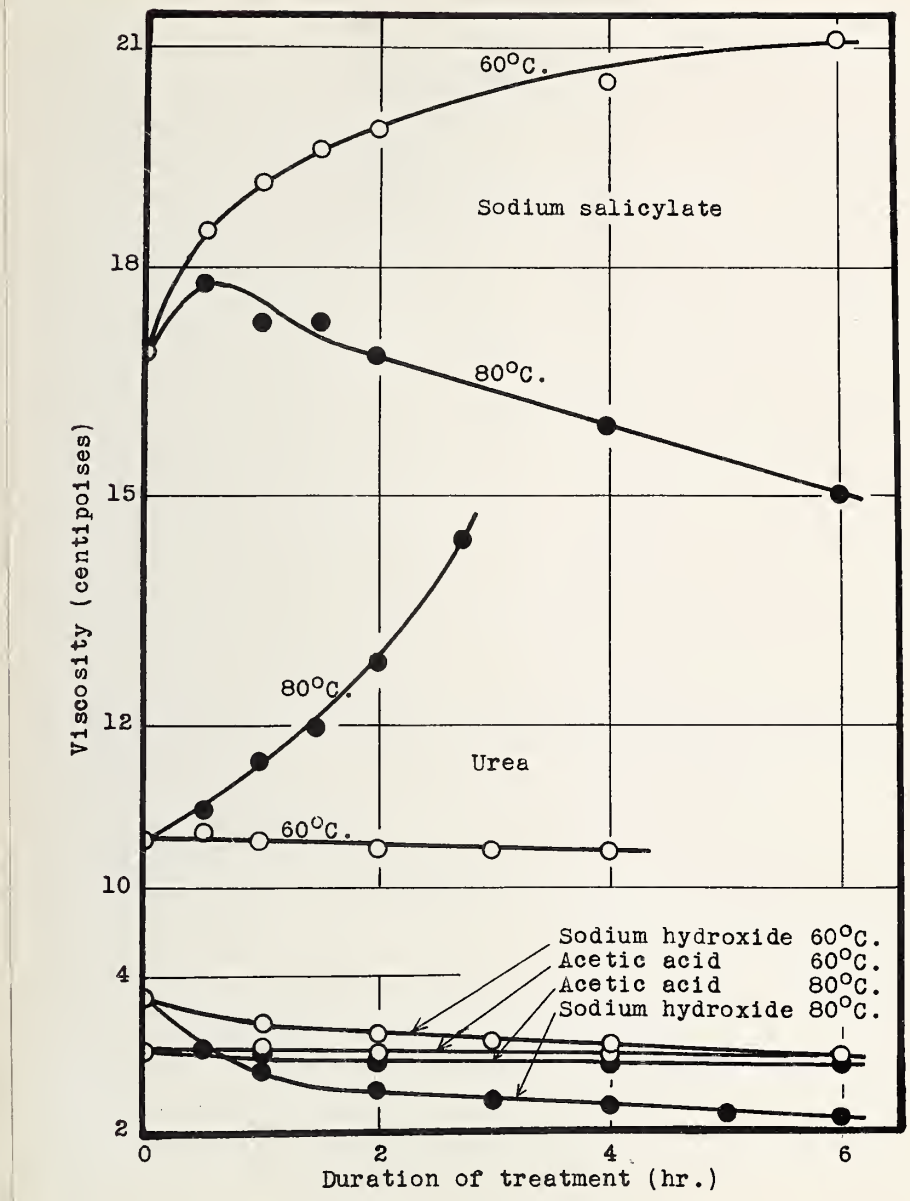

Fig. 6 - Effect of heat treatment on the viscosity of concentrated dispersions.

The viscosity behavior of dilute and concentrated dispersions in urea solution when heat treated at 80°C. is quite different. The viscosity of the dilute dispersion decreased, whereas that of the concentrated dispersion increased on heat treatment at 80°C. The dilute dispersions in the other reagents behaved essentially the same as the concentrated ones. This is evident when the curves in Fig. 5 are compared with the corresponding curves in Fig. 6. Judging from the results in Table XXIX, evaporation probably affected the viscosity of all the heat treated dispersions. Unfortunately, except in this one experiment, the effect of evaporation was not determined.

The nature of the viscosity decrease. Salting out tests were made on a dispersion in buffered 30% urea solution containing 4.52 mgm. of protein nitrogen per gram of dispersion. When necessary the pH was adjusted by adding concentrated ammonium hydroxide. The dispersion was 2 days old when heat treated. The results in Table XXXIV show the effect of heat treatment on the amount of nitrogen that can be precipitated from 10 cc. of dispersion with 2.75 cc. of 20% magnesium sulphate (anhydrous) solution.

Table XXXIV

Effect of heat treatment on subsequent precipitation of protein

Treatment	Viscosity	Viscosity as % of initial viscosity	pH of dispersion		Nitrogen precipitated (duplicates)	
			Before adjusting	After adjusting		
	centipoise	%			mgm.	
None	3.99	100.0	6.83	7.16	14.0	16.8
9.5 hr. at 40°C.	3.91	98.0	6.88	7.15	13.8	11.2
2 hr. at 70°C.	3.58	89.7	7.11	7.11	23.5	23.7
1 hr. at 80°C.	3.51	88.0	7.20	7.20	26.2	26.1

These results indicate that treatment at 40°C. decreased the amount of protein precipitated by the method adopted, although the difference cannot be considered significant. Treatment at 70° and 80°C. increased the amount of protein precipitated by this method, but whether this was due to dehydration or coagulation cannot be stated.

The viscosity change in dispersions in 30% urea solutions. The curves in Fig. 4 suggest an equilibrium reaction, as the final viscosity depends on the temperature of heat treatment. It was impossible to calculate the reaction constant at each temperature by means of the usual formulae, as η_∞ could not be obtained. The following method was employed. It was assumed that the same viscosity level, such as 90% of the initial viscosity, represented the same internal condition regardless of the temperature of treatment. The t values in Table XXXV were substituted

in the equation $K = \frac{1}{t} \ln \frac{a}{a - x}$, where a = the initial viscosity or 100%, and a - x = the viscosity expressed as a percent of the initial viscosity at time t. The K values, the Q_{10} values and the average Q_{10} value for each temperature interval are also given in Table XXXV.

Table XXXV

Values of t, K and Q_{10} calculated from heat treatment data

Temperature of treatment °C.	Viscosity as percent of initial viscosity 97.5	95.0	92.5	90.0	87.5	85.0	Av.
t values (min.). Obtained from Fig. 4.							
40	66	252					
60	24	66	150	360			
70	12	30	54	96	150	228	
80		15	27	42	66	96	
90			18	27	42	66	
100			12	18	30	48*	
$K \times 10^3$ values. $K = \frac{1}{t} \ln \frac{a}{a - x}$							
40	3.9	2.0					
50	6.0**	3.8**					
60	10.7	7.8	5.2	2.9			
70	21.3	17.2	14.4	11.0	8.9	7.1	
80		34.4	28.8	25.0	20.2	16.9	
90			43.2	39.0	31.8	24.6	
100			64.8	58.4	44.5	33.8	
Q_{10} values = $\frac{K_t + 10}{K_t}$							
Temperature interval							
40-50	1.5	1.9					1.7
50-60	1.8	2.1					2.0
60-70	2.0	2.2	2.8	3.8			2.7
70-80		2.0	2.0	2.3	2.3	2.4	2.2
80-90			1.5	1.6	1.6	1.5	1.6
90-100			1.5	1.5	1.4	1.4	1.4

* Value obtained by graphical extrapolation, Fig. 4.

** Values obtained by graphical interpolation, using the given K values in the respective columns.

It can be seen from the table that the Q_{10} varies with the viscosity level as well as with temperature. This indicates two opposing reactions and means that the original assumption, that the same viscosity level represented the same internal condition, regardless of temperature, was probably incorrect. The data are not adequate enough to permit extrapolating and determining the exact temperature at which each of the opposing reactions terminate, but the combined effect of the two gives a maximum Q_{10} in the region of 60° to 70°C., with one reaction predominating at lower and the other at higher temperatures. Owing to the wide variations between individual values (especially in the 60° to 70°C. interval) the average Q_{10} values may not be as significant as they appear to be.

It is true that, as the reaction temperature increased, the average pH at which the reaction proceeded also increased. However, Lewis (23, 24) found that the velocity of heat denaturation of oxyhemoglobin and of egg albumin was minimal at about pH 6.8, and unless the gluten proteins behave quite differently than the proteins that Lewis studied, the pH change could not account for a maximum Q_{10} value.

The results of other experiments also favor the suggestion of the existence of two reactions. The salting out tests on dilute dispersions show that storage at 25°C.

and heat treatment at 40°C. decreased the ease of precipitation, (Tables XVI and XXXIV), while treatment at 70° and 80°C. increased the east of precipitation, (Table XXXIV). Furthermore, the heat treatment at 60°C. of concentrated dispersions in 30% urea solutions decreased the viscosity, whereas at 80°C. it increased the viscosity (Table XXVI).

Until further studies are made, the nature of the reactions cannot be determined.

DISCUSSION

In the first part of this investigation it has been shown that the viscosity of gluten dispersions depends on the dispersing agents employed and the storage conditions. In all cases the viscosity of dispersions in acid and alkali reached a much lower level on storage at 25°C. than did that of dispersions in the neutral solvents. This indicates that acids and alkalis either dehydrate the particles or break up the aggregates more completely than do the neutral solvents. In this respect acids and alkalis might be considered better dispersing agents than the neutral reagents. McBain (28) stated that the best solvents for nitro-cotton are those in which dismemberment proceeds furthest through solvation of the various points of affinity. Acids and alkalis, however, also cause

incipient hydrolysis or molecular disintegration which is undesirable from the standpoint of a study of the physical properties of proteins. Salting out tests on dilute dispersions in urea solutions indicate that the viscosity decrease on storage at 25°C. is due to a breaking up of the aggregates forming the micellar structure, but as the viscosity remains at a relatively high level urea solutions apparently do not carry this process nearly as far as do acids and alkalis. The viscosity of dilute dispersions in urea solutions stored at 0°C. after 8 hours at 25°C. remained essentially constant. The effect of heat treatment at 80°C. (Part II) on the viscosity of a dilute dispersion in urea solution was essentially the same when the dispersion had been kept at 25°C. for 8 hours as when it had been stored at 0°C. for 4 days. It appears, therefore, that urea causes little further change at 0°C. after dispersion is complete.

In the second part of this investigation it has been shown that the viscosity of dilute and concentrated dispersions in acid and alkali always decreases on heat treatment of the dispersion, whereas that of dispersions in the neutral solvents may increase in certain cases. The magnitude of the viscosity changes of the dispersions in acid and alkali is much smaller than of those in the neutral solvents.

The viscosity of dispersions in sodium salicylate solutions increases on heat treatment of the dispersion at 60°C. At 80°C. the viscosity first increases and then decreases. The viscosity of dilute dispersions in urea solutions decreased at all the temperatures at which heat treatment was carried out. On the other hand that of concentrated dispersions decreased on heat treatment of the dispersion at 60°C. and increased on heat treatment at 80°C.

Computation of the Q_{10} value for the viscosity changes of dilute dispersions in urea solution shows that it reached a maximum between 60° and 70°C. This and other evidence leads to the suggestion that there are two reactions effecting the viscosity changes observed. At the lower temperatures there is probably a continuation of the reaction noted at 25°C., that is, a further disintegration of the aggregates. Above 70°C., however, a second reaction which increases the viscosity sets in. Salting out tests indicate that dispersed gluten which has been heat treated at temperatures above 70°C. is more easily precipitated than dispersed gluten which has not been heat treated. The second reaction must therefore be regarded as a dehydration or coagulation, and since the latter would account for the increase in the viscosity of concentrated dispersions it appears to be the more probable.

As denaturation of the coagulable proteins is generally accompanied by an increase in viscosity, it appears that the behavior of gluten in the neutral solvents is more akin to the behavior of albumin in water than is that of gluten dispersed in acids and alkalis. Denaturation proper is generally regarded as being chemical in nature and is followed by coagulation which is a physical phenomenon. As the chemical changes are not generally capable of detection, coagulation must be regarded as the first evidence of denaturation. The results presented in this paper show no evidence of the coagulation of dispersions in acids and alkalis.

Although the present data do not warrant a definite conclusion regarding the best dispersing agent for gluten, it appears that the neutral solvents are less drastic in their action.

CONCLUSIONS

The method developed for determining the protein nitrogen content of dispersions in 30% urea solutions has been found satisfactory.

When dilute gluten dispersions in 30% urea solutions are stored at 0°C. after initial periods at 25°C. there is little further change in the viscosity

of dispersions that have been stored at 25°C. for 8 or more hours.

When dispersions are stored at 25°C. the magnitude and behavior of the viscosity of dilute and concentrated dispersions in urea, sodium salicylate, acetic acid and sodium hydroxide solutions depend on the dispersing agent used.

Varying the pH of dilute dispersions in urea solutions from 6.1 to 8.0 had no appreciable effect on the viscosity.

The Q_{10} value for the viscosity changes of dilute dispersions in urea solutions reached a maximum between 60° and 70°C. This and other evidence lead to the belief that there are two reactions effecting the viscosity changes observed in the dispersions in urea solutions.

When dilute and concentrated gluten dispersions in urea, sodium salicylate, acetic acid and sodium hydroxide solutions are heat treated at 60° and 80°C. the viscosity behavior of the dispersions depends on the dispersing agent, the concentration of the dispersion and the temperature of heat treatment.

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